BIO_11 - Antigen binding evaluation of anti-*Acinetobacter baumannii* monoclonal antibodies

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Introduction: Healthcare-associated infections, along with increased antimicrobial resistance, is considered a global public health problem. *Acinetobacter baumannii* is an opportunistic bacterium related to hospital infections that result mostly in pneumonia associated with mechanical ventilation. The fact that *A. baumannii* has a broad spectrum of resistance to all antibiotics commonly used in the clinic, including polymyxin, a last resort antibiotic due to its potential for toxicity, has limited the treatment of infections caused by this pathogen. In 2017, *A. baumannii* resistant to carbapenemic antibiotics was classified by the World Health Organization as a pathogen of critical priority indicating the need for research and development of new antibiotics. In this scenario, non- traditional antibacterial agents, such as monoclonal antibodies (mAbs), emerge as a promising approach with higher specificity.

Objectives: To evaluate the binding and affinity of two anti-*A. baumannii* mAbs to the recombinant or native target antigen.

Methodology: Two mouse hybridoma secreting anti-*A. baumannii* protein mAbs were previously selected, and the mAbs were purified by protein A affinity chromatography. Fluorescence microscopy evaluated the binding ability of mAbs to the bacterial surface of a non-capsulated *A. baumannii* strain (AB307.30). Equilibrium dissociation constant (Kd) of each mAb to the recombinant target protein was determined by Isothermal Titration Calorimetry (Nano ITC) technique to assess binding affinity.

Results: Previous ELISA and Western Blot assays demonstrated that both mAbs can specifically recognize the recombinant target protein. The mAbs were also able to recognize the native protein in bacterial lysates of different *A. baumannii* strains, which indicated that the target protein is conserved. Here, fluorescence microscopy demonstrated that there is a specific protein recognition site to the mAbs in the intact bacterial surface. Moreover, the Nano ITC assay presented a 1:1 ratio, for both antibodies, that is one antibody binding site per protein. Likewise, both mAbs showed an affinity for the recombinant target protein, with a higher affinity to mAb 1 (Kd = 8.79 nM) when compared to mAb 2 (Kd = 35.76 nM).

Conclusion: Both mAbs were successful in demonstrating target-binding ability and high affinity. However, other functional tests are in progress and animal model tests will be performed to evaluate the antibacterial activity of mAbs. Considering the results, these mAbs showed potential for immunotherapy and immunodiagnosis of *A. baumannii* infections.

Keywords: monoclonal antibodies, antimicrobial resistance, bacterial infection